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OBSERVATIONS ON THE FINE STRUCTURE OF THE FIBROBLAST FROM A CASE OF EHLERS-DANLOS SYNDROME WITH THE MARFAN SYNDROME*

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Light and electron microscopic studies of the dermis from patients with the Ehlers-Danlos syndrome have shown that the dermis is thinner than normal and that dermal collagen is decreased in amount although morphologically (1), tinctorially, and histochemically it appears normal (2). Furthermore, no structural or quantitative alterations have been reported in the fibroblasts of these patients. Although elastic fibers have been observed to be increased (3–9), it is generally accepted that this is relative to decreased amounts of collagen and probably not the primary site of the anatomical defect in this disorder. Despite the fact that fibroblasts in patients with Ehlers-Danlos have been reported as structurally comparable to those in normal controls (2), there is ample clinical evidence that these patients have impaired healing. This is characterized by delayed wound healing and the development of thin, noncontracting, supple scars (10). In the light of these observations it was the purpose of this study to compare the fibroblasts in the healing wound of a patient previously reported with both Ehlers-Danlos and Marfan's syndrome (11) with those in wounds of normal individuals of comparable sex, age and race. Since impaired healing is not known to be a recognized part of Marfan's syndrome, it was felt that its coexistence with Ehlers-Danlos would not invalidate a study of the fibroblast in the latter condition.

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MATERIALS AND METHODS

Biopsies of skin, including dermis, were removed from the right upper chest of a nineteen year old Negro male with classical features of Ehlers-Danlos and the Marfan syndrome. The biopsy site was closed with silk sutures and rebiopsied ten days later.

Three healthy Negro males between eighteen and twenty-two years of age served as controls and were subjected to identical procedures. Tissues for light microscopic study were fixed in 10 per cent buffered formalin and embedded in paraffin. Sections were stained with hematoxylin and eosin, and the Mallory trichrome stain. Fragments of tissue (0.5 to 1 mm) for electron microscopy were fixed for 1.5 hours at 4° C in osmium tetroxide buffered to pH 7.4 with phosphate buffer (12). These tissues were prepared in the routine fashion and embedded in maraglas (13). Sections 1 μ thick were stained with toluidine blue for light microscopy (14) and thin sections cut from appropriate blocks were stained with uranyl acetate (15) and lead citrate (16) and examined in a RCA EMU-3F electron microscope.

RESULTS

The uninjured skin and dermis of the patient obtained at the first biopsy was indistinguishable from that of the controls. Paraffin sections of granulation tissue from the control wounds contained large, elongated fibroblasts with pleomorphic and intensely basophilic nuclei and moderate amounts of extracellular fibrillar material (Fig. 1A). In contrast, sections of wound tissue from the patient showed numerous, small, rounded fibroblasts with less intense nuclear basophilia and a paucity of extracellular fibrillar material (Fig. 1B). Under the electron microscope control granulation tissue consisted of fibroblasts in which the cytoplasm contained a highly organized endoplasmic reticulum composed of many membrane-lined cisternae with large numbers of attached ribosomes. The extracellular com-

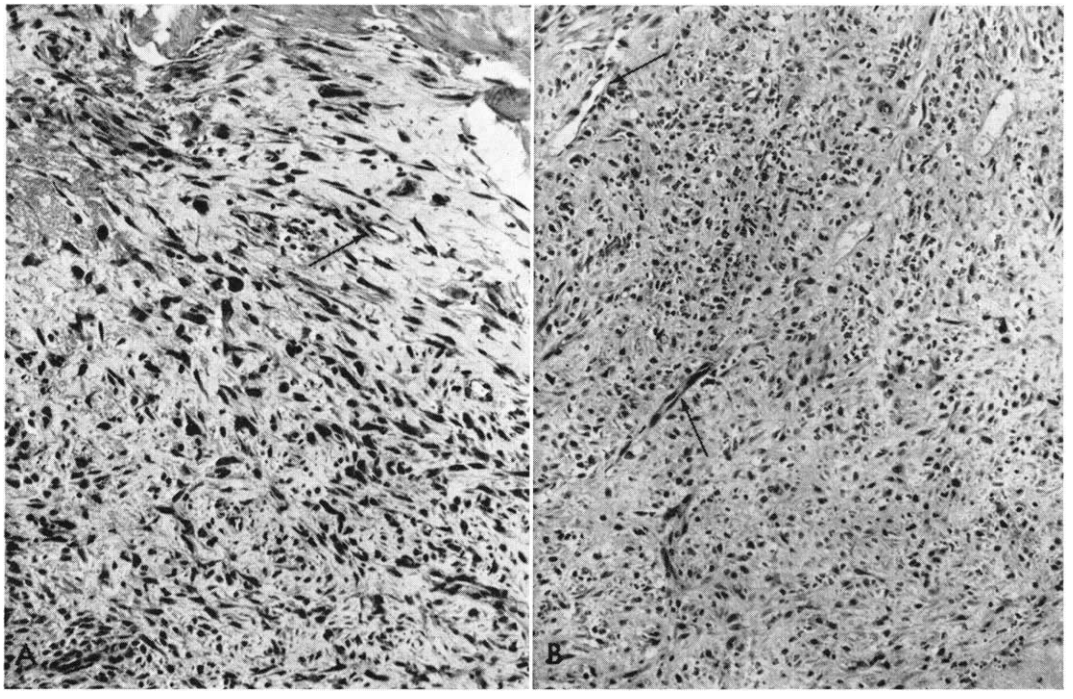

FIG. 1A. Granulation tissue from the healing wound of a normal 22 year old Negro male 10 days after the initial biopsy. The fibroblasts are large and exhibit pleomorphic and intensely basophilic nuclei. A capillary (arrow) is shown for comparison of magnification with Fig. 1B. Hematoxylin and eosin stain. × 170.

FIG. 1B. Granulation tissue of comparable age to that shown in Fig. 1A from the healing dermal wound of a 19 year old Negro male with the Ehlers-Danlos and Marfan syndrome. The fibroblasts, though numerous, are smaller and exhibit more rounded and less basophilic nuclei than fibroblasts shown in the previous figure. Several capillaries are evident (arrows). Hematoxylin and eosin stain. × 170.

partment contained large bundles of collagen fibers (Fig. 2A). At the level of ultrastructure the majority of fibroblasts in the granulation tissue from the patient exhibited cytoplasm in which the endoplasmic reticulum consisted of few cisternae lined by membranes associated with ribosomes and the ground cytoplasm was more dense than that present in the control fibroblasts. The extracellular compartment contained small and sparse bundles of collagen and a finely fibrillar material (Fig. 2B). At higher magnification it was evident that the dilated cisternae of the endoplasmic reticulum of control fibroblasts were filled with an amorphous material and that the Golgi complex was very well developed. Mitochondria were round and exhibited the membranous structure characteristic of these organelles (Fig. 3A). The endoplasmic reticulum of fibroblasts from the patient contained few dilated cisternae and many of the ribosomes were either free or in polysomal clusters, rather than bound to membranes. The mitochondria were often swollen and occasionally contained whorls of tightly packed dense membranes (Fig. 3B). Abnormal configurations which appeared to be derived from cytoplasmic membranes were also regularly encountered in the endoplasmic reticulum of these fibroblasts (Fig. 4). The extracellular finely fibrillar material was devoid of any recognizable periodicity. Collagen fibers which were present exhibited a range of periodicity from 620–650 Å, well within the accepted 640 Å value for normal collagen.

DISCUSSION

Despite numerous morphologic studies of the cutaneous lesion of the Ehlers-Danlos syndrome, the nature of the underlying defect is still a matter of controversy. McKusick (10) postulates that the error may lie in the molec-

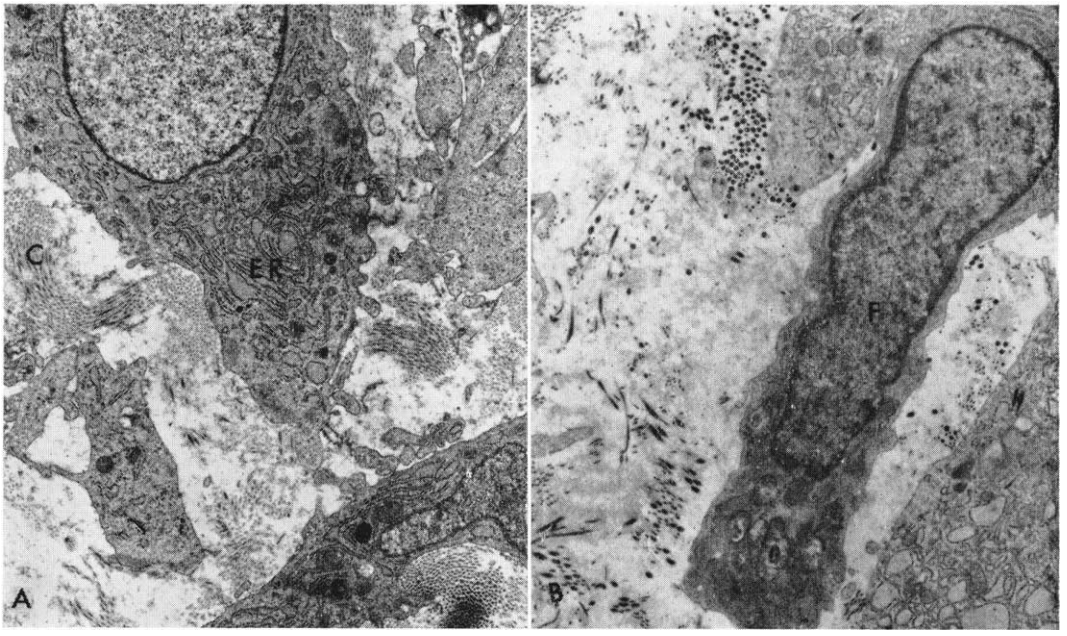

FIG. 2A. Fibroblast in the granulation tissue of a normal subject. The cytoplasm consists largely of profiles of rough surfaced endoplasmic reticulum (ER). Note the numerous dense bundles of collagen fibers (C) in the extracellular compartment. × 8,500.

FIG. 2B. Fibroblast in the granulation tissue of the patient with the Ehlers-Danlos syndrome. The endoplasmic reticulum of the fibroblast (F) appears poorly developed and is virtually devoid of rough-surfaced endoplasmic reticulum. A profile of cytoplasm of a more normal appearing fibroblast is visible in the right lower corner of the photograph. The extracellular compartment contains few collagen fibers, and material of low electron density. × 8,500.

ular structure of collagen, a view in keeping within light microscopic studies in which the continuity (17, 18) and organization (3, 19) of collagen was described as abnormal. Others contend that since the ultrastructure of collagen in Ehlers-Danlos is indistinguishable from the normal, the defect is not one of molecular organization (2).

The results of this study, though preliminary, suggest that the defect in Ehlers-Danlos may reside in the fibroblast. Since the endoplasmic reticulum is the cell organelle in which the majority of protein synthesis occurs (20), alterations of this structure in fibroblasts in healing wounds of patients with Ehlers-Danlos may be interpreted as presumptive evidence in support of a defect in the synthesis of collagen. Implication of this cytoplasmic component as the locus of the defect is not inconsistent with the decreased dermal collagen, deficient hydroxyproline content, and lowered thermal stability of collagen observed in this disease (21). It is noteworthy in this regard that simultaneous autoradiographic and electron microscopic study of the biosynthesis of collagen by normal fibroblasts utilizing tritiated proline suggest that collagen is synthesized in dilated cisternae of the endoplasmic reticulum and resides in the Golgi elements prior to secretion extracellularly (22, 23). More recent experiments by Ross and Benditt (24) indicate that precursor collagen protein may pass directly from the endoplasmic reticulum into the extracellular compartment without mediation of the Golgi complex.

It is of interest that in their experimental study of wound healing in scurvy, Ross and Benditt (25) encountered ultrastructural alterations of the endoplasmic reticulum in the fibroblast consisting of a dissociation of ribosomes from the membranous component. The alterations were reversible upon the administration of ascorbic acid. Furthermore, in the scorbutic state they also observed extracellular accumulations of finely fibrillar material

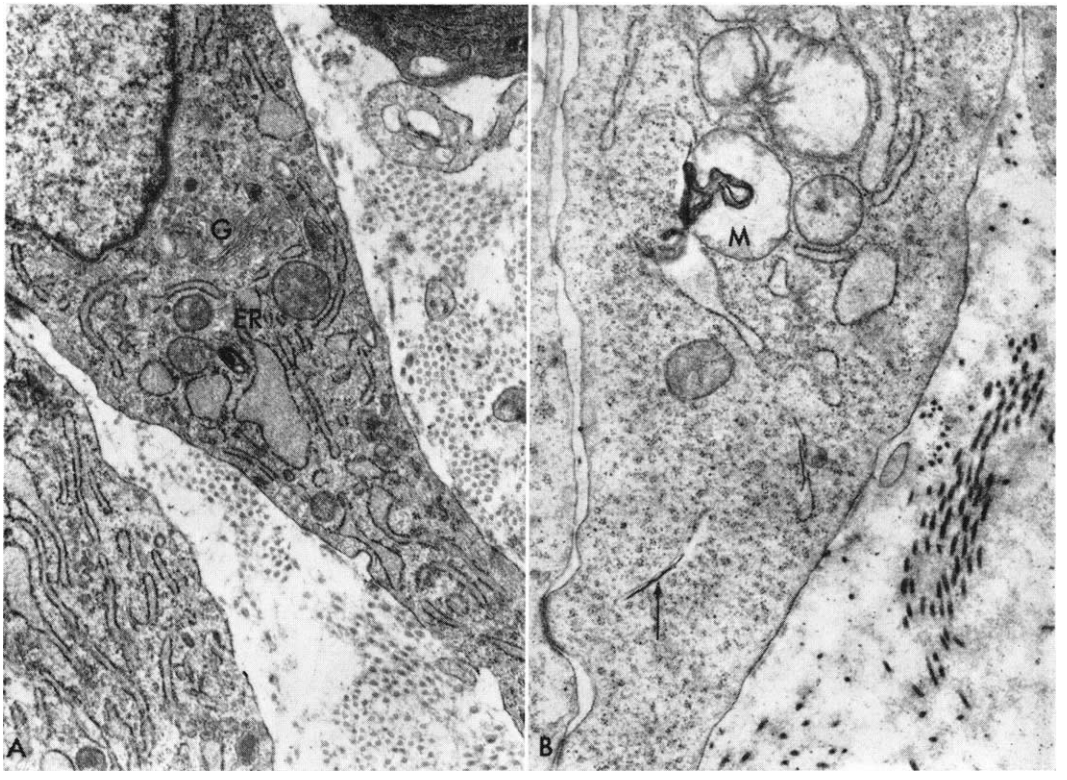

FIG. 3A. A higher magnification of a fibroblast in the granulation tissue of a normal subject. The cytoplasm contains the highly structured endoplasmic reticulum (ER) consisting of dilated cisternae filled with dense amorphous material characteristic of a stimulated fibroblast. The Golgi complex (G) with its numerous flattened sacs, and vesicles, is evident in one of the cells. × 20,500.

FIG. 3B. A fibroblast in the healing wound of the patient with Ehlers-Danlos showing a paucity of rough-surfaced endoplasmic reticulum, numerous polysomal clusters of ribosomes, and dense membrane-like inclusions both in the endoplasmic reticulum (arrow) and a swollen mitochondrion (M). At this magnification the extracellular material appears to consist of masses of small thin fibrils. × 20,500.

similar in appearance to those seen in the healing wound of our patient with the Ehlers-Danlos and the Marfan syndrome. In the healing wounds of scorbutic animals, this material disappeared following the administration of ascorbic acid and preceding the appearance of collagen fibers. Although it may be related to deficient synthesis of collagen, the origin and nature of the extracellular material remains obscure. The possibility that this material may be implicated in the extracellular formation of collagen is strengthened by the fact that it is seen to accumulate in two conditions of dissimilar etiologies in which there appears to be impaired production of collagen.

According to Wechsler and Fisher (2) no ultrastructural abnormalities were encountered in the dermal fibroblasts of patients with the Ehlers-Danlos syndrome. It may be that the cytoplasmic defects became manifest only in fibroblasts which have been stimulated to proliferate and synthesize collagen, as in the present instance. The duality of fibroblasts with respect to their synthetic activity has been stressed by Chapman (26), and more recently Dumont (27). There may be clones of fibroblasts in patients with the Ehlers-Danlos syndrome which are incapable of responding to the stimulation incident to injury. The fact that not all fibroblasts in the present case exhibited structural cytoplasmic abnormalities fits well with the genetic phenomenon of expressivity which determines the ex-

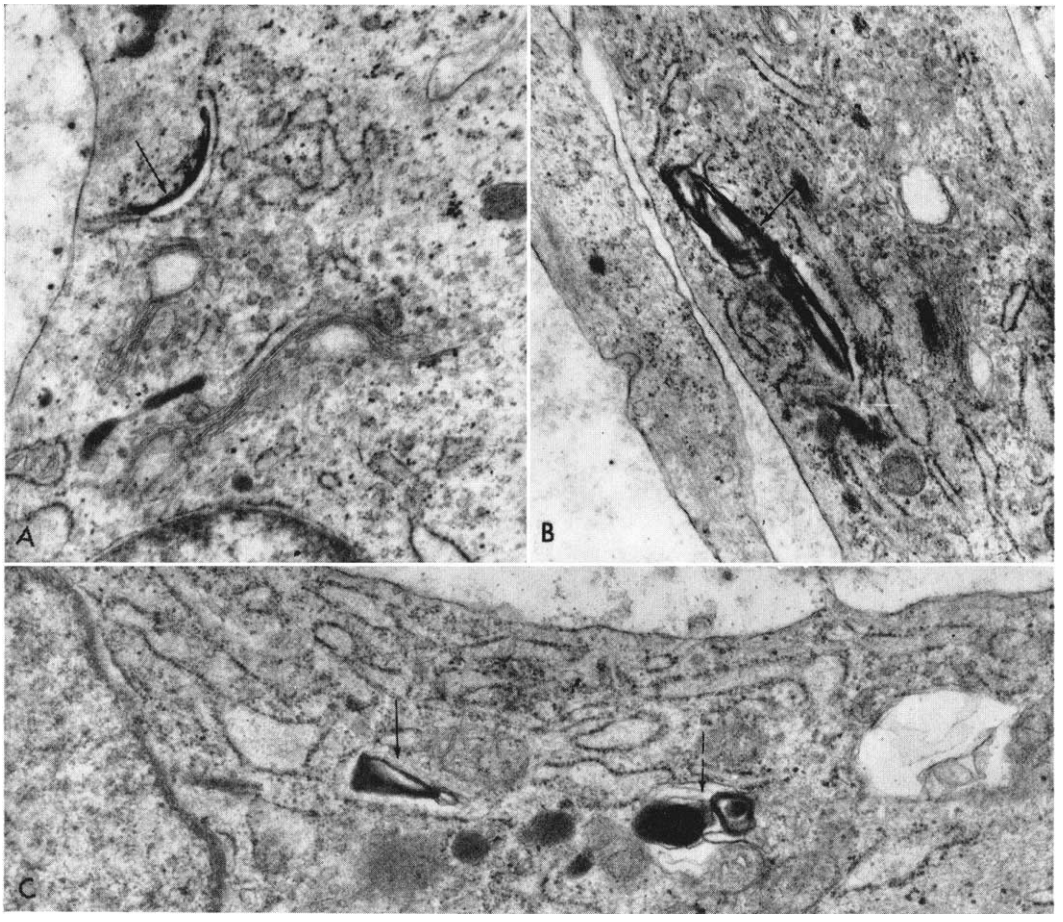

FIG. 4. Typical examples of membrane-like inclusions (arrows) encountered in the endoplasmic reticulum of fibroblasts of the patient. In 4A, a number of free ribosomes are visible in the right half of the photomicrograph. 4A × 24,000; 4B × 28,000; 4C × 28,000.

tent of the phenotypic expression of a mutant gene.

The deficient development of the endoplasmic reticulum, and focal injury of the endoplasmic reticulum and mitochondria encountered in the fibroblasts of the present case lead us to speculate that these may represent the morphologic manifestations of the subcellular lesion or lesions eventuating in impaired collagen synthesis in the Ehlers-Danlos syndrome. However, final proof of this must await chemical studies of the fibroblasts in this condition in which the abnormal cytoplasmic components are isolated and the nature of the defect determined.

SUMMARY

A morphologic study of granulation tissue from a 10 day healing dermal wound was performed in a patient with the Ehlers-Danlos and Marfan's syndrome. Two distinct populations of small fibroblasts were encountered at the level of ultrastructure. One resembled the classical fibroblast in which the cytoplasm consists of a highly organized rough-surfaced endoplasmic reticulum. The other type of fibroblast showed a cytoplasm in which there was a paucity of rough-surfaced endoplasmic reticulum and the majority of ribosomes were either in polysomal clusters or free. The extracellular compartment contained small and sparse bundles of collagen and an unidentified fibrillar material of low electron density. These findings were in contrast to those encountered in three controls of comparable race, sex and age, in which identical wounds showed large fibroblasts, all of which contained abundant rough-surfaced endoplasmic reticulum consisting

of dilated cisternae filled with material of moderate electron density. The extracellular compartment contained abundant and large collagen fibers and no material of low electron density.

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